PHYSIOLOGICAL STUDY OF MAPLE SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 260

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(WITH TWO FIGURES)

Introduction

The appearance of two taxonomic species within the same genus is not always a criterion of similar physiological or ecological behavior. The seeds of two closely related species, as those of the sugar and river maples (*Acer saccharum* Marsh. and *A. saccharinum* L.), show a striking contrast in season of maturity, reaction to external conditions, chemical composition, and in their physiological behavior in general. The sugar maple matures its seeds in the fall, and these must pass through a well defined period of after-ripening before germination can take place. The storage substances are mainly protein and fat, with a small amount of carbohydrate present. On the other hand, the river maple ripens its seeds in the spring. The seeds germinate almost immediately upon a moist substratum, but if allowed to desiccate for some time under ordinary atmospheric conditions they soon lose their power of germination. A very small percentage of fat and protein is present, starch being the chief storage product.

It is a matter of common observation that many mature seeds and spores soon lose their power to germinate when subjected for varying periods to atmospheric desiccation. In a great many tropical seeds death follows atmospheric drying. In our own region the seeds of the willow and cottonwood are usually cited as the classic examples of death due to desiccation shortly after seed fall. The cottonwood gives low percentage of germination and low seedling vigor after two weeks of desiccation in laboratory air, while after three weeks seeds fail to germinate when placed in the most favorable germinative conditions. Cottonwood seeds, however, are in a high state of metabolic activity when first shed.

At 30° C. on moist filter paper the fresh seeds will usually give 100 per cent germination within 24 hours. The hypocotyls will attain a length of 8–9 mm., and the cotyledons will be entirely spread. SCHRÖDER (**23**) states that seeds of *Caltha palustris* failed to germinate after 11 weeks of storage over sulphuric acid and after 20 weeks of storage in the ordinary atmosphere. DELAVAN (**8**), working with the oaks and hickories, concludes that a cold even temperature, although the atmosphere be moist, is better than warm dry storage of seed. Seeds of *Oxalis*, elm, river maple, hornbeam, birch, beech, chestnut, and probably many others have their germinative power lowered or lost entirely by varying periods of desiccation.

Heretofore no work has been done on seeds, sensitive to drying, regarding the exact or approximate water content at the time of death. Furthermore, it has never been demonstrated whether loss of viability is due in part to temperature or entirely to desiccation effects.

Investigation

RIVER MAPLE (*Acer saccharinum* L.)

In the Chicago region *Acer saccharinum* matures its seeds the latter part of May or early in June, varying with the season. At the time of fall the seeds contain approximately 58 per cent of water, being almost fully imbibed. The seeds soon germinate if they lodge upon a moist substratum, but if they are subjected to desiccation there is an immediate reduction of the moisture content, and their viability is lost long before an air-dry condition is attained. The seeds of the river maple were chosen for this study because they are large, making it possible to obtain material readily in sufficient quantities for chemical analysis. The period of time between maturing and loss of viability is of moderate duration, permitting a study of internal changes accompanying desiccation; also seeds are abundant and easily collected. In all cases where reference is made to the maple fruit the seed plus the ovary wall is taken into consideration. Seed refers to the embryo plus the integuments. In all storage conditions the entire maple fruit was used; this holds for both the river and sugar maple. The criterion

for the beginning of germination is the protrusion of the tip of the hypocotyl through the integuments.

Water and temperature relations

Fruits were collected at time of shedding and stored at various constant temperatures from 0 to 40° C. At 25° C. and above fruits were stored in open wire baskets. At 20° C. and below they were stored in loosely covered cans which contained a considerable quantity of calcium oxide. The lime facilitated drying at the lower temperatures, besides preventing the accumulation of an excess carbon dioxide pressure about the seeds. By August 26, 1918, all seeds desiccated at 0–40° C. had lost their

TABLE I

LIFE DURATION OF SEEDS STORED AT VARIOUS DRYING TEMPERATURES

Storage temperature	Life duration*
35° C.	6 days
30	8
25	22
20	20
10	49
0	92

*At 25° C. the humidity of the air was considerably higher, and drying somewhat slower than at 20° C., accounting for increased life duration.

ability to germinate. In all cases seeds were considered to have lost their viability when 80 per cent failed to germinate when placed on moist filter paper at 30° C., all seeds having either germinated or decayed. From 0 to 35° C. the seeds lost their viability when the water content was reduced to 30–34 per cent. So far as could be determined, the various temperatures from 0 to 35° C. for desiccation do not appear to raise or lower the critical point of water content. At 40° C. death does not seem to be due to desiccation. Seeds turn black in a short time, killing apparently being due to the destructive action of this high temperature. One apparent effect of increasing temperatures (0–35° C.) is the shortening of the desiccation period, no change being evident in

the percentage of water at several temperatures at the time of loss of viability.

Seeds have a high metabolic activity at time of fall. Where viability and vigor are so closely allied with high water content, it is logical to suppose that the initial vigor can be retained for some time by holding the water percentage at the initial content, and by lowering the metabolic activity. Seeds at maturity and for some time thereafter give off considerable amounts of CO_2. For a number of samples at time of fall the yield of CO_2 was estimated as approximately 7 mg. per gram of dry weight per 24 hours at 25° C. If we consider 7 mg. as the amount of CO_2 respired in 24 hours at 25° C., the seeds would soon exhaust their store of food if the initial activity were maintained. The carbohydrate present would be entirely exhausted and the seeds die of starvation within approximately 120 days if this initial intense respiratory activity were maintained. At this rate it would be impossible to hold seeds just below the point of saturation at the higher temperature for any great length of time. Seeds, however, can be held for some time stored over water at low temperatures. Seeds harvested in the spring of 1917 were stored over water in desiccators at 10° C., and continued to give 95–100 per cent germination until November 1917. There was, however, an abnormal development of the hypocotyl during the latter part of the storage period at 10° C. No alkali was placed in the desiccators to prevent CO_2 accumulation, so it is impossible to say just what part was played by the carbon dioxide in the preservation of the seeds at this temperature. In the spring of 1918 seeds were stored over water in a large desiccator at 0° C. A bottle of strong alkali was also placed in the desiccator to prevent accumulation of a CO_2 blanket. These seeds were discarded after 102 days' storage, and at this time seeds were giving 100 per cent germination. They had retained their initial vigor and appeared to be normal in every respect. Perhaps many other seeds of this general behavior would retain their viability and vigor for considerable periods when placed in similar storage conditions. Seeds can be kept for a considerable period at temperatures just below the freezing point. After 50 days seeds stored at −5° C. gave good germination. At this low temperature care

must be taken that water does not come into contact with the outer walls of the fruit or integuments, as ice formed on the latter appears to inoculate the subcooled tissue below, and freezing to death results.

Respiration

Respiration was determined on newly collected seeds, on seeds desiccated at 25° C., and on germinating seeds. Determinations were made on the desiccating seeds every second day until viability was lost, and for several weeks thereafter. All respiration experiments were conducted at 25° C., as this temperature was thought to correspond very closely with the average temperature to which the seeds would be subjected under natural conditions. The method of determining the carbon dioxide given off was that described by GRAFE (12), with slight modifications. In general the method consists in pulling carbon dioxide free air over the respiring material through a column of barium hydroxide. The barium hydroxide solution is held by a Reiset tube. The air is drawn through slowly and uniformly. This is accomplished best by the air replacing water which is slowly siphoned out of a large demijohn by means of a capillary tube. At the end of a determination the barium carbonate was allowed to settle and an aliquot part (25 cc.) of the 100 cc. of barium hydroxide was pipetted off and titrated with N/20 oxalic acid. Phenolphthalein was the indicator used.

If the intensity of respiration may be used as a criterion of metabolic activity, then the seeds of the river maple at time of fall are in high state of metabolism. In the desiccating seeds there is a fall the first few days in respiratory activity, and then a gradual rise until a maximum is reached. This maximum is retained for several days, then there is a gradual decline, until only a trace of carbon dioxide is given off. This secondary rise in respiratory intensity may accompany increased starch hydrolysis. It will be seen later that accompanying desiccation there is a great increase in sucrose, due to starch hydrolysis. The later fall in respiratory activity is probably caused by a deficiency of water. The greatest respiratory activity was obtained on the desiccating seeds with a water content of approximately 44 per cent. There is no marked

degeneration of the respiratory enzymes during this fall, because when dead seeds are placed in germinative conditions the respiration again mounts to a high value, giving off 8.84 mg. of carbon dioxide per gram of dry weight in 24 hours. It is not known, however, just what percentage of the carbon dioxide given off in the latter case was due to bacterial action. HAAS (13) found that the marine alga *Laminaria*, in the presence of certain reagents, respired more rapidly after death than in the living condition. MAIGE and NICOLAS (17) have done considerable work on respiration in correlation with the state of turgidity of certain plant organs,

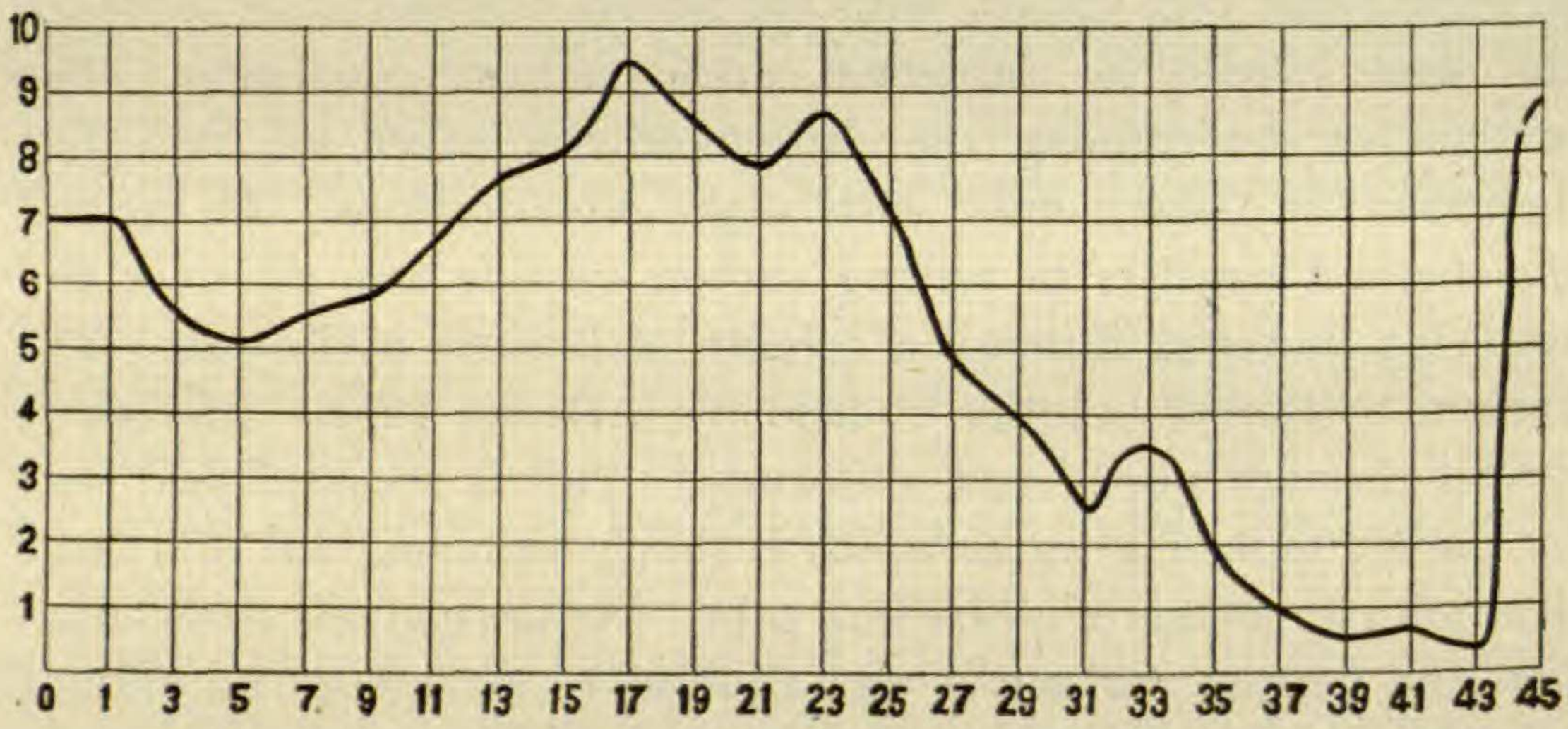

FIG. 1.—Respiration curve for seeds desiccating at 25° C.; mg. of CO_2 given off in 24 hours per gm. dry weight plotted on ordinates; time of desiccation in days plotted on abscissae; great rise in respiration after forty-third day due to placing desiccated seeds (dead at time) under favorable germinative conditions.

as buds, leaves, and embryos. They find in material taken directly from the tree increased carbon dioxide production with increased turgescence, also for decreased turgescence, and usually an increase in respiration when decrease was followed by an increase. Fig. 1 represents the trend of respiration during 43 days of desiccation. The sudden rise on the forty-fifth day shows respiratory activity of seeds after being placed in germinative conditions.

To determine the respiratory activity of germinating seeds, newly collected seeds were planted in the dark at 25° C. The respirometer used was a 500 cc. graduated cylinder. This was half filled with shredded filter paper, previously well sterilized. The

filter paper was packed very loosely in the graduated cylinder. The seeds were washed with distilled water and planted near the surface of the paper, about midway between the top and bottom of the chamber. A small amount of water was run into the respirometer. The top was stoppered and supplied with an inlet tube which extended to the bottom of the chamber and brought in the carbon dioxide free air, and with an exit tube which carried the carbon dioxide laden air to the Reiset tube. The seedlings were grown in the dark and consequently there was no food manufactured. Storage food only was used up in respiration.

The respiratory activity of the germinating seeds reaches a maximum about the eighth day at this temperature. At this time the seedling has elongated considerably, the radicle having attained a length of 7–10 cm., varying considerably with the individual. After the eighth day respiration decreases gradually. Seeds stored for several weeks at a low temperature (0° C.) and then transferred to a high temperature (25° C.) in germinative conditions show a very high initial respiratory intensity, which soon drops to normal, and then again increases. PALLADIN (**20**) found that transferring the tips of etiolated bean seedlings from a lower to a higher and also from a higher to a lower temperature increased the respiratory activity. According to APPLEMAN (**1**), tubers stored at low temperature for several weeks and then transferred to room temperature respire more intensely than tubers of the same lot not subjected to the cold storage conditions. He thinks this increased respiration might result from the increased accumulation of sugar at the lower temperatures.

Fig. 2 shows the march of respiration during the first 14 days of germination in the dark. In general this curve agrees with that found by RISCHAWI (**21**) for the respiration of the wheat seedling growing in the dark, but is quite different from that found for the bean.

Catalase activity

The apparatus used for catalase determinations was a modified form of the one used by APPLEMAN (**2**). Determinations were made upon fresh seeds, seeds desiccating at 25° C., and also seeds germinating in the dark at 25° C. Entire seeds were used in all cases.

Material was weighed, then ground in a mortar with a small amount of quartz sand and a knife point of calcium carbonate for exactly 2 minutes. This emulsion was then washed with the aid of 10 cc. of distilled water into a 200 cc. wide-mouthed bottle. The latter was then corked and plunged into a water bath kept at 25° C. The commercial form of Oakland dioxygen was used at all times. This dioxygen gives an acid reaction. To neutralize the acidity a small excess of calcium carbonate is added to the dioxygen just

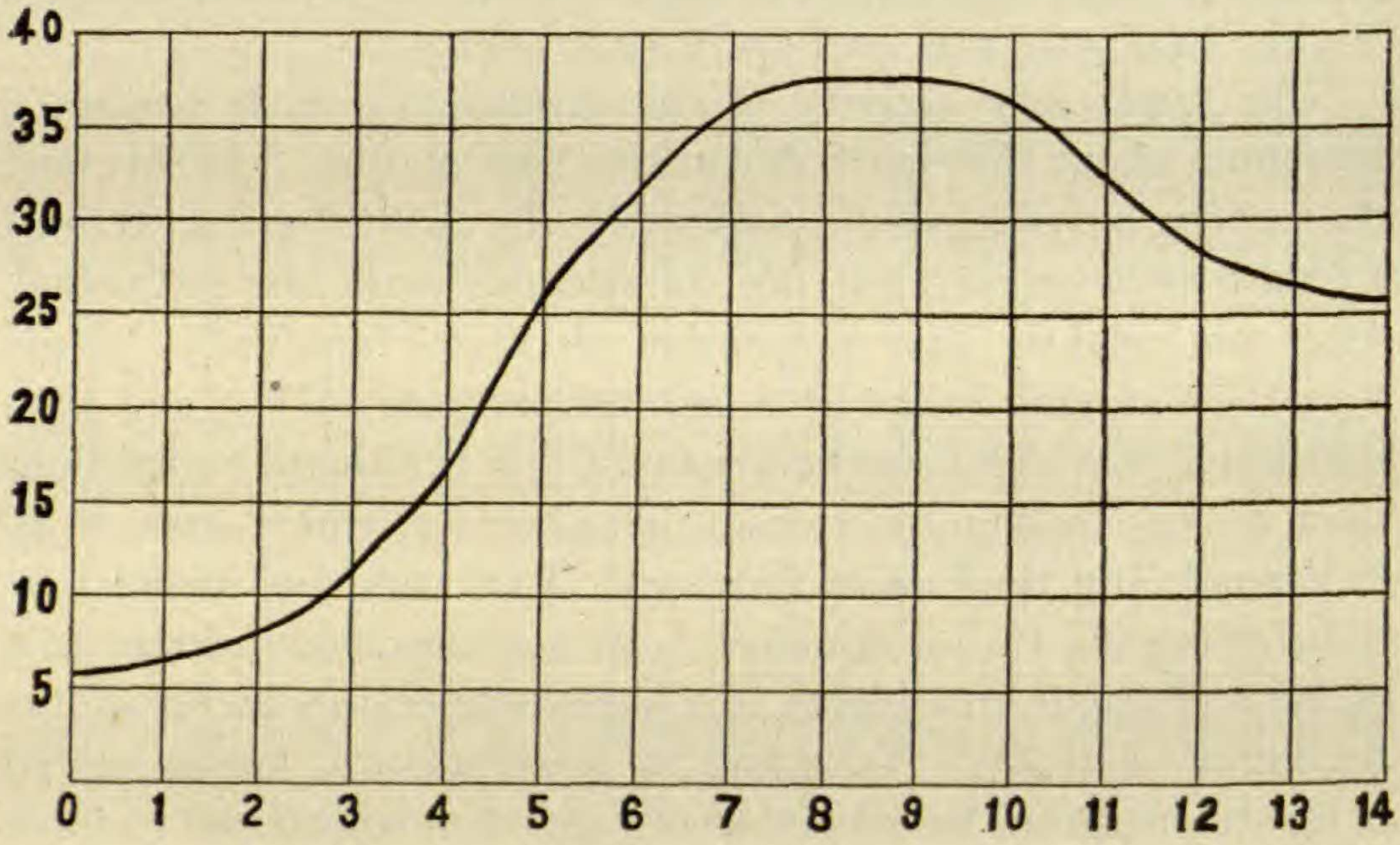

FIG. 2.—Respiratory curve for first 14 days of germination in dark at 25° C.; time of germination in days plotted on abscissae and mg. of CO_2 given off in 24 hours per gm. of dry weight plotted on ordinates.

before using. If the acidity is not corrected, the catalase activity is reduced approximately one-half. A small separatory funnel inserted in the cork of the bottle holds the dioxygen. The latter is run into the ground tissue when the dioxygen and pulp have reached the same temperature as the water bath. The material is then shaken uniformly for 10 minutes by means of a small motor. The oxygen liberated is collected over water at atmospheric pressure in a 100 cc. burette. Table II shows the catalase activity at various times during desiccation and the early stages of germination.

Catalase activity increases slightly during the first few days of desiccation, but decreases gradually thereafter. This activity seems to align itself in a general way with respiratory activity, which remained high for a considerable time. With germination the catalase activity increases enormously, appearing to be closely correlated with metabolic activity. There is not a sudden drop in the catalase activity at the time of loss of viability, as one might

TABLE II

CATALASE ACTIVITY ACCOMPANYING DESICCATION AND FIRST STAGES OF GERMINATION

CONDITION OF SEEDS OF SEEDLINGS	NO. OF CC. OF O_2 GIVEN OFF BY 1 GM. OF DRY WEIGHT IN	
	5 minutes	10 minutes
Fresh seeds collected May 25 (1918)	952	1248
Desiccated at 25° C. for 3 days.....	1035	1373
" " " " 5 "	1106	1447
" " " " 10 "	977	1341
" " " " 14 "	1075	1359
" " " " 18 "	1022	1259
" " " " 22 "	868	1098
" " " " 26 "	731	979
" " " " 34 "	688	909
" " " " 42 "	461	593
Desiccated in laboratory for 8 months..	380	500
Seedlings with radicle 1 cm. long.....	1245	1565
" " " 2 " "	1717	2055
" " " 3 " "	2106	2566
" " " 4 " "	2438	3060
" " " 5 " "	3216	4472

expect, but a gradual decrease correlated with respiratory activity and water loss. After a storage for 8 months under laboratory conditions the catalase activity was reduced more than one-half below that of the fresh seed.

Oxidase and peroxidase

Peroxidase activity is very intense in the fresh seeds. A dark blue color is obtained immediately upon addition of alcoholic solution of benzidine and a drop of dioxygen. As desiccation progresses there is a gradual decrease in peroxidase activity. In one-year-old dead seeds there is only a very pale blue color evident

about the vascular tissue when this method is used. No oxidase could be detected by the ordinary qualitative chromogenic methods in either the living or desiccated seeds.

Chemical analysis

In the following analysis seeds were collected from the same tree in order to eliminate differences due to individual variation. The collection was made in the spring of 1917. Fresh seeds were immediately placed in 95 per cent redistilled alcohol, enough being added to make the final volume of alcohol 80 per cent. One-half gram of calcium carbonate was added to guard against possible acid hydrolysis. In the final calculation the calcium carbonate was considered as being in the insoluble fraction. In general the method of extraction and analysis is that outlined by KOCH (16), but a few modifications were found necessary.

TABLE III

Fraction	Fresh seeds	Desiccated seeds
Percentage F_3 of total dry weight ..	79.05	65.56
" F_2 " " " " ..	15.8	30.31
" F_1 " " " " ..	5.15	4.13

The tissue was ground, and then extracted with hot 95 per cent alcohol for four hours, followed by 1-hour ether extraction. The alcohol-ether insoluble material was then heated in water for one hour on the steam bath. The water was evaporated down, alcohol again added, and returned to extraction cups for a 24-hour alcohol extraction and 1-hour ether extraction. The alcohol and ether extracts were combined, evaporated to dryness, and then extracted with anhydrous ether. This ether extract is known as F_1; the residue from the ether extract is F_2; the alcohol-ether insoluble material is F_3. F_3 was dried in the oven at 103° C. for 5 days, then cooled and weighed.

The 1917 seeds were desiccated in the laboratory. No attempt was made to maintain a constant temperature. The seeds failed to germinate after 18 days, when the water content had dropped to approximately 34 per cent. The desiccated seeds were treated in the same manner as the fresh seeds. Table III shows the

percentage variation in the various fractions accompanying desiccation.

It can readily be seen that accompanying desiccation under laboratory conditions there is a great increase in F_2. One would be led to expect quite the contrary, as condensation is quite commonly associated with desiccation in plants. Table IV shows more in detail to what this increase is due.

During the period of desiccation there has been an enormous increase in the percentage of sucrose. Accompanying this increase

TABLE IV

ANALYSIS OF FRESH AND DESICCATED SEEDS

MATERIAL	PERCENTAGE TOTAL DRY WEIGHT	
	Fresh seeds	Desiccated seeds
Free reducing sugar	0.53	0.43
Sucrose (calculated as invert sugar)	4.53	14.41
Starch	48.18	35.42
F_1 Nitrogen	0.03	0.02
F_2 Nitrogen	0.65	0.80
F_3 Nitrogen	3.36	3.28
F_1 Phosphorus	0.03	0.02
F_2 Phosphorus	0.18	0.31
F_3 Phosphorus	0.50	0.35

is a corresponding decrease in the starch content. Free reducing sugars remain approximately the same. In the desiccated seeds we also find a slight increase in phosphorus and nitrogen in F_2. The nitrogen here represents merely the Kjeldahl nitrogen.

SUGAR MAPLE (*Acer saccharum* Marsh.)

Historical

A very different type of behavior is found when the seeds of the sugar maple are considered. Germination here is initiated by a distinct period of after-ripening. Investigators generally have used the term "after-ripening" as referring to the series of chemical or physical changes occurring within the embryo or associated structures, which bring to a close the dormant period and make germination possible. The factors operating to cause delayed germination in most types of seed dormancy studied to the present

time have been treated in some detail by CROCKER (5). Seeds that have dormant periods fall naturally into two groups: (1) seeds, like certain members of the Leguminosae, have embryos capable of immediate germination, but dormancy is here induced by associated structures like the seed coats or pericarp; (2) the embryo itself may be the cause of delayed germination. The second type of dormancy may be due either to an immature embryo, as found in *Ceratozamia* (4) and *Ilex opaca* (14), the former often being shed at the time of or shortly after fertilization, while in the holly the embryo is merely a globular undifferentiated group of cells at the time of seed fall; or dormancy may appear in apparently fully matured embryos, as is the case in some members of the Rosaceae. The seeds of the sugar maple fall into the latter group, having a dormant, morphologically mature embryo.

DAVIS and ROSE (7) found that in nature *Crataegus mollis* has a dormant period of a year or more. This period of dormancy can be shortened considerably by removing the carpel and testa. It is doubtful whether any such interrelation exists between the embryo of the sugar maple and its inclosing structures.

The sugar maple sheds its fruit in the fall, after the first few hard frosts. When given the most favorable conditions for germination at time of fall the seeds fail to respond. The seeds must be kept at a low temperature, with plenty of moisture present for a considerable period of time for after-ripening to reach completion. Under natural conditions, if the seeds are kept moist during the fall and winter, after-ripening will be complete the latter part of February or early part of March.

Investigation

The object of the investigation was twofold: (1) to determine the optimum temperature and water relations for after-ripening; and (2) to determine the changes taking place within the embryo during the after-ripening period. The fruit of the sugar maple was collected the latter part of September and early part of October direct from the trees in the Chicago region and northern Indiana. Fruits were stored dry in wire baskets at various temperatures from -5 to $+30°$ C.; others were stored in desiccators over water at

5° C. and 10° C.; also, some were stored out of doors on the surface of the ground and kept covered during the fall and winter to prevent drying.

Temperature and water relations

When seeds were stored dry, in no case, regardless of storage temperature, did after-ripening reach completion; that is, no dry stored seeds would germinate when placed in Petri dishes on moist cotton at favorable germination temperatures. All dry stored seeds required a prolonged stay at low temperatures with plenty of moisture present to completely after-ripen. DAVIS and ROSE found that after-ripening in the haw proceeded best at temperatures near 5° C. The sugar maple was also found to after-ripen best at about this temperature.

In January, after three and a half months of dry storage, specimens were removed from each of the dry stored samples, and placed at 5° C. under good germinative conditions. The pericarp was removed and the seeds that had been dry stored at 5° C. were the first to complete their period of after-ripening, most of the seeds completing after-ripening during the fifth week. The seeds, however, do not after-ripen uniformly; some precede and others follow the general average time. Seeds dry stored at −5° C. take the longest time to complete their period of after-ripening, taking 4–5 weeks longer than seeds dry stored at 5° C. Seeds dry stored at 10–30° C. after-ripen more slowly than seeds stored at 5° C., and more quickly than seeds stored at −5° C. In other words, seeds dry stored at 5° C. have progressed farthest, and those stored at −5° C. have progressed least in the process of after-ripening at their respective storage temperatures. The factor limiting the complete after-ripening in the dry stored seeds at low favorable temperatures is a deficient water supply. Only in the presence of sufficient water can the various processes go progressively on to complete after-ripening.

Fruits stored on the surface of the ground were subjected to the temperature ranges of the soil surface. The seeds, however, were kept saturated, due to the extremely wet fall and winter. At time of fall seeds had a water content of 55 per cent, and during the entire fall and winter the water content remained at 55–57 per

cent. In the seeds stored out of doors and in desiccators over water there was no indication of increased water holding capacity accompanying after-ripening. Seeds stored in desiccators at low temperatures over water are completely after-ripened several weeks before seeds stored out of doors. Table V shows how after-ripening progressed in seeds stored out of doors. As after-ripening progressed, less and less time was required for the completion of this process when placed in the germinator at 10° C.

TABLE V

Put to germinate at 10° C.	Percentage of germination after number of days indicated											
	1	2	3	4	5	6	8	12	17	26	30	35
January 16, 1918	....	....	....	....	....	....	....	....	....	....	68	88
February 4	....	....	....	....	....	....	....	39	83	92	....	....
February 28	19	50	....	....	....	....	92	....	....	....	....	....
March 5	40	67	77	85	95	97	100	....	....	....	....	....

Seeds after-ripened out of doors and at 5° C. are more vigorous than seeds after-ripened at slightly higher temperatures (10° C.). Dry stored seeds at low temperatures are more vigorous when after-ripened than seeds previously dry stored at high temperatures. This question of vigor should be given more attention than it has been given up to the present time. There is something very significant in the fact that maximum vigor can be obtained by after-ripening seeds at a temperature so much below the optimum germination temperature and at a temperature which we consider retarding to metabolic activity in general. Poor germination and high seedling mortality can be replaced by good germination and vigorous seedlings when the most favorable temperature (about 5° C.) and water relations are used for after-ripening. After-ripening and germination is a continuous process, but the optimum temperature for germination is considerably above the optimum for after-ripening. Seeds completely after-ripened at 5° C. are stimulated to very rapid growth when placed at higher temperatures. On the other hand, if seeds are completely after-ripened and then allowed to desiccate at higher temperatures, seedling vigor is lowered as time progresses, and in several weeks the

embryo fails to respond when placed in favorable germinative conditions. The reason for this loss of vigor is not known. It may be due to the increased respiration, using up the plastic substances essential for the initiation of germination, or to the introduction of some new factor inhibitory to growth. After-ripened seeds placed at $-5°$ C. and kept saturated by packing in snow will retain their initial vigor for a considerable time.

Oxygen pressure

The most favorable oxygen pressure for after-ripening was not studied in detail. Seeds after-ripened in desiccators are under considerably reduced oxygen pressure. The oxygen is soon used up in respiration. Nevertheless, these seeds stored at a low constant temperature will after-ripen quicker than seeds stored out of doors with a good supply of oxygen, but subjected to fluctuating temperatures. Seeds stored in open baskets, but kept saturated at low constant temperatures, will after-ripen sooner than those stored in desiccators, and the resulting seedlings appear to be more vigorous.

Oxidase and peroxidase

ECKERSON (11) found an increase in oxidase and peroxidase activity accompanying after-ripening in the haw. In the peach CROCKER and HARRINGTON (6) found no increase in oxidase activity in the after-ripening seeds when ordinary chromogens or the Bunzel methods were used, but the pulp of the after-ripened seeds exposed to air shows a more rapid oxidation of its own chromogens. In the sugar maple there is a slight increase in peroxidase activity accompanying after-ripening, being more pronounced in the hypocotyl. No oxidase could be detected in dormant or after-ripened seeds when guaiaconic acid or benzidine was used as a chromogen.

Catalase

One of the most consistent phenomena accompanying the after-ripening of this type of embryo is the increase in catalase activity. This increase is continuous, increasing manyfold during the early stages of germination. ECKERSON (11) found that catalase activity increased in the haw with after-ripening. In

Tilia ROSE (**22**) also found a noticeable increase in catalase activity accompanying after-ripening. CROCKER and HARRINGTON conclude that "seeds that after-ripen in a germinator at low temperatures (commercial layering), in which the dormancy of the embryo is self imposed and the embryo experiences fundamental time-requiring changes for after-ripening, show a great increase in catalase activity with after-ripening (*Crataegus*, *Tilia*, *Prunus*)."

Catalase determinations were made upon the dormant and after-ripened seeds and upon the seedlings at various stages of germination. In all cases the integuments were removed and a definite number rather than a definite weight of seeds was used. The material was weighed and samples were run as described for the soft maple. The after-ripened seeds and also the seedlings used were after-ripened and germinated in the dark at 10° C. Table VI demonstrates the great increase in catalase activity accompanying after-ripening and germination in seeds of the sugar maple.

TABLE VI

STAGE	CC. OF O_2 LIBERATED BY 1 SEED OR SEEDLING IN		CC. OF O_2 LIBERATED PER GM. OF DRY WEIGHT
	5 minutes	10 minutes	10 minutes
Dormant	23.4	31.1	754
After-ripened	33.7	39.3	1117
Seedlings with 1 cm. radicle	31.0	37.0	1058
" " 2 " "	51.0	60.4	1716
" " 3 " "	87.2	98.4	2235
" " 4 " "	99.7	114.0	2230
" " 5 " "	89.2	107.0	2786
" " 6 " "	113.2	130.0	4481
" " 7 " "	125.0	142.5	4440

An increase in catalase activity is evident in both cotyledons and hypocotyl. Seeds germinated at higher temperatures also gave slightly increased catalase activity when taken at the same stage of development. Seedlings with radicles 1 cm. long were used to determine the relative catalase activity of the different parts. One-tenth gram (wet weight) of radicles, cotyledons, and integuments liberated in 10 minutes 95, 43, and 5.1 cc. of oxygen respectively. The hypocotyl, which is the most actively growing

organ at this time, gives by far the greatest catalase activity. The storage organs (cotyledons) give considerable catalase activity. The inert structures (integuments) give very low catalase activity. The difference here would be still more striking if calculated as percentage of dry weight. CROCKER and HARRINGTON find the catalase activity of wheat embryo 28–29 times that of the endosperm. The same investigators find that in grass seeds in general the physiologically inactive organs show only a small fraction of the catalase activity shown by the embryo.

Dry dormant seeds stored in the laboratory were used to determine the Q_{10} for catalase activity at temperatures ranging from 10° C. to 50° C. Seeds were ground very fine and rubbed through a 100-mesh sieve. One-tenth gram samples were used for determinations. Ten cc. of dioxygen, 10 cc. of water, and a small excess of $CaCO_3$ were added to the meal. Table VII shows the Q_{10} value for catalase activity.

TABLE VII

TEMPERATURE	Q_{10} FOR		
	1 minute	5 minutes	10 minutes
10–20° C.	1.4	1.3	1.3
20–30° C.	1.3	1.2	1.1
30–40° C.	0.1	0.9	0.8
40–50° C.	0.8	0.6	0.5

In no case does the van't Hoff law, which calls for an increase of 2–3-fold for every 10° C. rise in temperature, hold. The time consumed in heating the sample to the higher temperature introduces considerable error. The time required for complete destruction of catalase activity at any given temperature was not determined. There was still some catalase activity at temperatures slightly above 50° C. APPLEMAN (2) found the catalase activity in potato tubers to be entirely destroyed at 50° C. Between 0° C. and 10° C. he finds the Q_{10} for catalase activity to be 1.5. From 10° C. to 40° C. he gets lower Q_{10} values for potato catalase than was given by the catalase of the sugar maple.

Chemical analysis

Samples were analyzed as in river maple, with slight modifications to suit the material. One-tenth gram of $CaCO_3$ was added to samples at the time of collection. Figures in the tables represent averages from several samples. Dormant seeds had made no progress in after-ripening. It is almost impossible to choose seeds for the after-ripened samples that are known to be completely after-ripened. The only criterion for completion of after-ripening is germination. The seeds in the after-ripened samples vary from completely after-ripened ones to seeds probably within a week or 10 days of complete after-ripening.

TABLE VIII

Stage	Sugar calculated as percentage to total dry weight		
	Free reducing sugar	Sucrose (as invert sugar)	Polysaccharides (as glucose)
Dormant	0.06	6.40	5.21
After-ripened	0.67	4.32	4.66
Beginning germination, radicles about 1 cm	1.81	2.36	3.43
Seedlings with 2–3 cm. radicle (with integuments)	1.13	1.80	5.91
Seedlings with 5–6 cm. radicles (integuments shed)	0.06	2.62	5.43

The protein content of the seeds is exceptionally high. The seeds contain 7.17 per cent of nitrogen or approximately 44.8 per cent protein, calculated on a dry weight basis. The embryo itself contains almost 50 per cent of protein. The nitrogen multiplied by the factor 6.25 was used to indicate the amount of protein present. The seeds contain about 17 per cent of ether extract and 11.5 per cent of total sugars. The ash percentage is relatively high, 5.87 per cent of dry weight, while 0.91 per cent of the total dry weight is phosphorus. Only a trace of free reducing sugar is present in the dormant seeds, but sucrose or sucrose-like sugars are present in considerable amounts. Table VIII shows the relative amounts of various sugars at time of dormancy, approximately complete after-ripening, and early stages of germination.

Accompanying after-ripening there is a considerable increase in free reducing sugars. Free reducing sugar reaches a maximum at the beginning of germination, and then diminishes as germination progresses. There is, no doubt, a considerable amount of sugar used up in respiration during the long after-ripening period in the germinator even at temperatures as low as 5° C. Whether the appearance of considerable amounts of free reducing sugars is merely correlated with after-ripening or is essential for the completion of after-ripening is not known. The formation of free sugars may be favored by cool uniform temperatures and high state of hydration of the embryo.

TABLE IX

Stage	Kjeldahl nitrogen as percentage of total dry weight in		
	F_1	F_2	F_3
Dormant	0.03	1.58	5.56
After-ripened	0.03	1.48	5.59
Beginning germination, radicle about 1 cm	0.03	1.63	5.29
Seedlings with 2–3 cm. radicle (with integuments)	0.03	2.37	4.73
Seedlings with 5–6 cm. radicle (integuments shed)	?	3.15	4.94

Seedlings with radicles 2–3 cm. long show an increase in polysaccharides, but a decrease in free reducing and sucrose or sucrose-like sugars. Correlated with this increase in polysaccharides is a considerable reduction in percentage of fat. The percentage of ether extract drops from about 17 per cent in the dormant and after-ripened seeds to slightly less than 14 per cent in the seedling with a radicle 2–3 cm. long. The fats in the early stages of germination are probably converted into sugar or sugar-like materials, as found in the haw by ECKERSON (11), in the sunflower by MILLER (19), and in the castor bean by DELEANO (9).

With germination there is the usual increase of the more soluble nitrogen of F_2. There is no significant change in relative nitrogen value of the dormant and after-ripened seeds. Table IX shows the relative amounts of nitrogen in the various fractions at different stages of the seeds and seedlings.

Respiration

A detailed study of respiration of the after-ripening seeds at the lower temperatures may help to interpret the metabolic activity accompanying after-ripening. Little work has been done on this phase up to the present time. Preliminary tests show very little respiration taking place in dormant air-dry seeds. When these seeds are soaked for 48 hours, however, and then transferred to the respirometer, the respiratory intensity jumps to approximately the same level as that of fully after-ripened seeds. Sufficient data have not been obtained to justify a full discussion of the correlation between after-ripening and respiration.

Hydrogen ion concentration

The gas chain method described by MICHAELIS (18) was used to determine the hydrogen ion concentration. Two embryos were used in each case. They were ground for 2 minutes with a small amount of pure quartz sand and 1 cc. of distilled water, and 5 cc. of distilled water was then added. This solution becomes more alkaline the longer it stands, so several readings were taken immediately and the average of these used. In both the dormant and after-ripened embryo we find a distinctly basic condition. The average of several samples shows a P_H value of 8.335 in the dormant seeds and a P_H value of 7.909 in the after-ripened seeds. Both are distinctly on the basic side of the neutral point. The hypocotyls of the dormant seeds gave a P_H value of 9.048, while that of the germinating seedlings with a 1 cm. hypocotyl gave a P_H value of 9.055. Seeds that had just started to germinate were used in the latter case, to be sure that the period of after-ripening had been completed. ECKERSON (11) found increased acidity in the hypocotyl of the haw with after-ripening. In working with *Tilia americana* ROSE (22) found increased hydrogen ion concentration with after-ripening. In the sugar maple the embryo is always basic, although the hydrogen ion may increase in concentration in the embryo when it after-ripens.

Discussion

To the present time little work has been done upon seeds that show in general the same type of behavior as found in the river

maple. Numerous observers have reported cases of seeds dying when subjected to atmospheric conditions for a short period of time. As to just what factors operate with desiccation to cause lowering of seedling vigor and early death we are still entirely ignorant. In the river maple temperature does not appear to determine the critical percentage of water loss. Death occurs at all ordinary temperatures (0–35° C.) when the percentage of water in the seeds has reached 30–34 per cent. Whether or not this will hold in general for other seeds of this type will not be known until considerably more species have been studied. In the desiccated seeds we find a noticeable increase in permeability, indicated by a large amount of sugar appearing in the substratum when placed in the germinator. The sugar makes an excellent medium for growth of bacteria and fungi, and in a few days the entire seed is completely decomposed. The fungi appear to be unable to attack potentially vigorous seeds. Whether increased permeability is the cause or the result of death is not known. Desiccation may coagulate or denature the protoplasmic proteins, increasing permeability and subsequent leaching, allowing an inroad for parasitic organisms. This type of seed stands in marked contrast to that type of seed which retains its viability best when stored in an air-dry condition. DUVEL (10) even recommends drying the majority of seeds in a vacuum or over sulphuric acid to insure good preservation. In fact, many seeds can be dried to constant weight without lowering viability or seedling vigor. KIDD (15) states: "In the case of certain rapidly deteriorating seeds (*Hevea brasiliensis*) the carbon dioxide naturally produced by respiration of the seeds in a closed flask rose to 40 per cent, and the pressure of this was found to be accompanied by a marked prolongation of vitality in the seeds. This prolonged vitality was far in excess of that reached with the present commercial method of packing these short-lived seeds for export." Where there is a rapid oxidation of food material due to high respiration, there is no doubt that narcotizing the embryo would result in greatly reduced metabolic activity. Whether or not high embryo vigor can be maintained in the river maple by narcotizing still remains to be determined. Storage at 0° C. over water, however, provides an excellent condition for the seeds of river maple.

Recent studies have thrown considerable light upon the behavior of seeds that require a definite time under certain favorable conditions to after-ripen a morphologically mature embryo. The major portion of the work up to the present time has been done upon various members of the Roseceae. No doubt seeds of this general behavior exist in many more of our plant families, especially among the uncultivated forms. Not until more work has been done upon a wider range of plants will it be known just how widespread this phenomenon is. The few species studied thus far by various investigators show remarkable similarity of behavior in several features accompanying after-ripening. There are five more or less specific changes, according to CROCKER and HARRINGTON (**6**), which are quite conspicuous in the constant way which they seem to accompany after-ripening in seeds of this type: (1) rise in vigor of seedling, (2) increase in amount of water absorbed, (3) increase in total acidity, (4) increase in catalase, and (5) oxidase activity.

When after-ripening is accomplished under the most favorable conditions of oxygen pressure, water relations, and temperature, seedling vigor is in all cases at its maximum. In the sugar maple, at least, seedling vigor can be judged only during the first stages of germination after the completion of the period of after-ripening. After-ripening, however, may complete itself under conditions not favorable for the greatest expression of seedling vigor.

ROSE found slight increase in acidity accompanying after-ripening in the seeds of *Tilia*. This was correlated with greater water holding capacity. In the haw (**11**) delayed germination of the embryo has been found to be due to a dormant hypocotyl. In the dormant seed this organ is slightly alkaline or neutral, but with after-ripening the hypocotyl becomes distinctly acid. Accompanying this increased acidity there is increased water holding capacity of the hypocotyl, along with increased activity of the enzymes. Here the hydrophilous colloids have a greater water holding capacity in a slightly acid medium. When the entire seed of the haw is considered, however, we find a slightly higher water holding capacity in the dormant than in the after-ripened seed. In the sugar maple the water holding power of the hypocotyl only was not determined. Considering the hydrogen ion concentration

found in the hypocotyl of the dormant and after-ripened seeds, one would hardly expect to find a change in the water holding capacity of the hydrophilous colloids. Determinations on the water content of entire seeds stored in favorable after-ripening conditions show that there is no change in the water holding capacity of the seeds as a whole.

One of the most consistent phenomena accompanying after-ripening in this type of embryo is the great increase of catalase activity. This appears to be an accompanying feature of more than ordinary importance. A large number of investigators in various branches of animal and plant physiology attempt to correlate catalase activity with metabolic activity in general. BURGE (3), by increasing the work of certain fowl muscles and consequently the respiratory and metabolic activity, has made the catalase activity increase enormously. In the castor bean DELEANO (9) found a rapid increase in catalase activity at the beginning of germination. A great increase in catalase activity accompanied germination in the sugar and river maples. In the fully imbibed seed of Johnson grass, CROCKER and HARRINGTON (6) found catalase activity paralleling respiration. This did not hold for seeds of the amaranth, however. In the potato, APPLEMAN (1) found respiratory and catalase activity closely accompanying each other. ECKERSON (11) found an increase in the catalase activity with after-ripening in the haw. An increase in catalase activity with after-ripening has also been reported for *Tilia americana* (22). In the sugar maple there was a 66 per cent catalase activity increase in the after-ripened seeds over that of the dormant seeds. Just how closely catalase activity and respiration parallel each other during the course of after-ripening has not yet been determined. From evidence at hand showing the almost universal correlation of these two phenomena we might reasonably expect to find respiration increase noticeably during the process of after-ripening. Respiratory activity should be determined continually throughout the entire period of after-ripening at the temperature and water relations most favorable for after-ripening. Preliminary respiratory determinations reported in this paper are not conclusive. The seeds were transferred from 5° C. to the 20° C. oven. This change

in temperature no doubt introduces changes which may possibly mask the real condition at the lower temperature.

Accompanying after-ripening in the sugar maple is an increase in the amount of free reducing sugars. Just how generally this occurs in this type of embryo is still unknown. Whether increase in amount of free reducing sugar is essential for the completion of after-ripening is problematical. Dormancy is probably due to a temporary suppression in the development of one factor or a group of factors essential for the normal functioning of the embryo in germination. It is impossible to select any one factor as the cause of dormancy in the embryo of the sugar maple at the present time. Whether any certain observed change in the embryo accompanying after-ripening is responsible for bringing dormancy to a close, or whether this change results merely from the conditions to which the embryo has been subjected, remains a question.

Summary

River maple

1. Seeds lose their viability when the water content is reduced to 30–34 per cent.

2. Temperature seems to play no part in determining the critical point of water loss. Higher temperatures only hasten the rate at which the point of desiccation is attained.

3. Respiratory activity in the desiccating seeds at 25° C. first decreases slightly, then rises to a maximum, then gradually falls to zero as desiccation progresses.

4. After a slight initial increase, catalase activity gradually decreases in the desiccating seeds. Catalase activity increases enormously during the early stages of germination.

5. Seeds of a river maple may be kept in a vigorous viable condition for a considerable period of time at low temperatures (0° C.) stored over water.

6. There is a gradual decrease in peroxidase activity accompanying desiccation.

Sugar maple

1. Seeds after-ripen best at temperatures near 5° C., with a good supply of oxygen and moisture.

2. With after-ripening the seeds show a considerable increase in free reducing sugars.

3. Catalase activity increases greatly with after-ripening and germination; there is also a slight increase in peroxidase activity.

4. Both the dormant and after-ripened seeds have a reaction that is distinctly alkaline; this holds for the hypocotyl as well as for the entire embryo.

5. Fully after-ripened seeds will remain in this condition for a long time if kept moist at $-5°$ C.

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